OBSERVATIONS ON BLOW FLIES; DURATION OF THE PREPUPAL STAGE AND COLOR DETERMINATION.

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The results of two main lines of experiment upon blow flies are recorded in the following paper. The first was concerned with the duration of the prepupal or migration stage of the larvæ and the conclusions may be summarized as follows:

The length of the prepupal period is determined by environmental rather than by hereditary factors and these factors are both complex and obscure. In general, dryness, cold, or agitation due to crowding, tend to prevent pupation, while change from dryness to dampness or the reverse, induces pupation. The prepupal stage may be extended for a long period, four months in one experiment (1912-*f*), in warm temperature without injury to the development of adult flies, which emerge from the pupæ in normal condition. Lack of opportunity for the larvæ to bury themselves does not inhibit pupation. Exhaustion of the food supply before the larvæ have attained full size has a tendency to produce undersized but normally formed flies. The causes producing misshapen and imperfectly expanded flies are more obscure, but may be in part due to drying of the pupæ. Delayed pupation in *Lucilia* larvæ is evidenced by a change from white to pink in the fat bodies, but in two genera of larger flies, *Cynomyia* and *Calliphora*, the white color is maintained although considerable shrinkage of the whole body occurs. There is no evidence that overfeeding delays pupation, but much evidence that larvæ will pupate immediately despite the fact that they have had abundant opportunity to overeat.[2]

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[2] From his studies on blow flies, Herms (Herms, Wm. B., '07, "An Ecological and Experimental Study of Sarcophagidæ with Relation to Lake Beach Debris," *Jour. Exp. Zool.*, IV., 1) reaches the conclusion that an optimum of development is attained by the larvæ after a certain period of feeding and that continued feeding after this has a tendency to delay pupation. In many of my cultures, however,

The second main line of investigation was concerned with coloration in the adult flies and showed results as follows:

The first color assumed by the adults after their integument has hardened is a deep purple which rapidly changes to dark metallic blue in the larger forms experimented upon, *Cynomyia* and *Calliphora*, becoming greenish after a short time in the males of *Cynomyia*. In *Lucilia* the purple passes rapidly into a bright green, which is later replaced by more or less bronze. The degree of this bronzing tendency is evidently of an hereditary nature as different strains vary in this respect and selection is here effective. The environmental factors, light and temperature, seem to have no effect upon the degree or rapidity of this process.

The experiments recorded in this paper were performed upon the various species of blow-flies common in New England, especial attention being given to the common green-bottle fly, *Lucilia sericata*.[1]

larvæ of *Lucilia sericata* and *Cynomyia cadaverina*, which have been allowed to feed on all the fish that they would eat, on attaining the migration stage, have pupated within two or three days and have emerged from the pupæ in due course despite the fact that they have had plenty of opportunity to overeat.

In Table IV., Series 1, Herms records the mean weight of larvæ of *Lucilia* at the end of a feeding period of 60–72 hours as 38.183 milligrams, from 256 counts. This weight is regarded as the optimum for development. Among this series were a number that delayed pupation. These comprise Series 7. When weighed (there were 64 individuals) the mean weight was 33.59 milligrams. Although this weight is lower than the mean of Series 1, *it is considered*, that at the time of migration it must have been higher and that the difference is due to loss of moisture during the extended migration period, which had up to the time of weighing been twelve days. At the end of twenty days in the larval period as against a normal of about six days, 39 of these larvæ pupated, the rest being dead. *It is assumed* "that these larvæ were beyond the optimum weight, and for this reason pupation was deferred."

[1] Experiments were performed upon *Lucilia sericata* Meig., *L. sylvarum* Meig., *L. cæsar* L., *Calliphora erythrocephala* Meig., *C. viridescens* Desv., *C. vomitoria* L., and *Cynomyia cadaverina* Desv. Nothing has been done with the Sarcophagidæ, s. str., the flies named being included in the Calliphorinæ of the Muscidæ. Herms groups them all under the Sarcophagidæ, stating that he follows Girschner in this matter; but Girschner includes Calliphorinæ as his second group and Sarcophaga, Dexia, etc., as his fourth group under the family Tachinidæ, dropping the name Sarcophagidæ. (Girschner, E., '93, "Beitrag zur Systematik der Musciden," *Berl. Ent. Zeits.*, XXXVIII., 3).

While the work of Herms is said to have been done on *L. cæsar*, this is probably not correct as this species is relatively infrequent as compared with *L. sericata*, and Herms's material was collected from fish put out where all flies had ready

I have frequently found in my cultures imperfectly formed pupæ, sometimes misshapen and sometimes of normal form but with a soft covering. These have given rise in a few cases to undersized but normally formed flies, which lived as long as the full-sized individuals. In the majority of instances, however, the flies emerging were of normal size insofar as the chitinous parts of the body were concerned, but the abdomens appeared shrunken, the wings expanded only imperfectly, and the pigmentation failed to take on its customary brilliancy, remaining dull and opaque. This type of fly has also frequently appeared from pupæ apparently normal. In the case of *Luciliæ* which vary from a brilliant metallic green or greenish blue to a bright copper color becoming duller with age, the imperfectly formed flies resembled senescent individuals, being a dull coppery red. In the other larger species which are normally dark metallic blue with pollen that varies in amount in the different species and in the different individuals of the same species, the imperfectly expanded flies were dark blue in color without polish. That these imperfectly formed flies were hindered from normal development by drying of the pupæ, while small flies are produced from underfed larvæ might seem a reasonable explanation were it not

access, no distinction being made between the species of *Lucilia*. The relative abundance of *L. sericata* in the vicinity of Boston is especially to be observed where flies are crowded about a food supply in large numbers in which case it is only rarely that *L. cæsar* is taken. I have collected thousands of flies in the vicinity of the garbage scow, Boston, and at meat near the Bussey Institution, Forest Hills, and have found that less than one per cent. have been *L. cæsar*, while *L. sylvarum* has never been taken in such situations. At a very short distance from the Bussey Institution, however, where flies are relatively much fewer, *L. cæsar* and *L. sylvarum* have equalled and even surpassed *L. sericata* in numbers. I have never taken them, however, in any abundance. It is possible that *L. cæsar* may be more abundant in the region of the Great Lakes where Herms's work was done.

Calliphora erythrocephala is common about Boston all through the summer months while *viridescens* and *vomitoria* are rarely seen except in the spring and fall. *Cynomyia cadaverina* occurs only in the spring and fall and seems entirely to disappear during the summer. That these variations in frequency are entirely due to temperature and that there is no necessary periodicity in the breeding habits of the flies is evidenced by the facts that the forms disappearing during the summer still continue to breed farther north and that any of the species may be bred throughout the year by the proper regulation of temperature conditions. *Luciliæ* and *Calliphoræ* have been bred throughout the winter at Cambridge by Dr. A. O. Gross and *Calliphoræ* and *Cynomyia* have been bred during the entire year at Forest Hills by myself.

for the fact that in many cases the full-sized imperfectly formed specimens have come from pupæ kept damp. Nor can this be due in all cases to drying of the larvæ in the prepupal stage as will appear from experiment 1912-*f* recorded below.

Rarely has there been any considerable prolongation of the pupal stage in my experiments. Eclosion has taken place in approximately the time expected varying slightly with the species and the temperature. In one case, however, a few pupæ of *L. sericata* were obtained which failed to emerge and were kept for a number of weeks in a warm room in damp sand. At the end of that period they were examined for the possible presence of parasites, but nothing of that nature was found. I have no explanation to offer for this unusual fact.

On August 8, 1912, a female specimen of *L. sericata*, 1912-*f*, was taken at the garbage scow, Boston. She laid a number of eggs in less than a week and the larvæ soon reached the migration stage. This occurred before August 20. They were then placed in dry sawdust at room temperature and shortly there appeared thirteen flies, four males and nine females. None of the other larvæ pupated and at various times they were removed and examined. They seemed to be in a normal condition and reacted negatively to light. It was observed that the general tint of the larvæ gradually changed from white to light pink due possibly to the exhaustion of nutriment in the fat bodies. On October 23 they were all very pink but were still active although they had been in dry sawdust at room temperature for at least two months. At this time they were placed in damp sand and pupæ were soon formed from which thirteen males and eleven females emerged from November 11 to November 19. On November 20 and 21, several more flies emerged but unfortunately these were not counted. By December 4 the sand had dried somewhat and an examination showed nine larvæ remaining. The sand was dampened and on December 24, two flies emerged; on December 31, two more; and on January 3, one fly came out. This was the last of the lot, the other four having been killed on December 25 and studied for the presence of parasites, such as bacteria, etc. Nothing of this sort was found, however.

We have here then four larvæ which survived in the prepupal stage for at least four months at room temperature. The experiment indicates that in this case dryness has been the cause of the delay.

From the pupæ formed by these larvæ, there emerged after the usual time, flies which were of full size and completely expanded. The only difference observed in them from flies having a short prepupal period was the fact that the abdomen was contracted in a dorso-ventral direction. This deficiency, however, was corrected after a few days of feeding and the flies lived for a normal period of time. An extended prepupal stage under warm and dry conditions does not then necessarily produce misshapen flies, which often appeared from pupæ formed directly after the feeding period of the larvæ and which were permanently deformed, having the abdomen distorted and shrunken laterally as well.

From this experiment I conclude that hibernation may be undergone in the prepupal period. It is my opinion also that the majority of hibernating flies pass the winter in this condition and that the moistening of the soil in the spring by the melting of snow and the rain induces them to pupate. Many of the flies appearing on warm days in winter probably come from pupæ which are apparently not hindered from eclosion except by extreme cold.

That drying is not the only cause for delayed pupation, I am led to believe from the fact that in many instances larvæ have refused to pupate when buried in sand of all degrees of moisture in my various cultures both of *L. sericata* and of *C. cadaverina*. While this prolongation of the larval stage was in some cases undoubtedly due to cold, this cause could not be assigned to all cases as they often refused to pupate in summer temperature in moist sand.

A female of *L. sericata*, 1912-*V*, was taken at the Bussey Institution on November 20, 1912. One lot of her larvæ comprising 151 individuals reached the migration stage on December 4 and 5. They were distributed in eleven glass jars, but since conditions were the same in some of the jars I have grouped them for convenience into five series which represent the five conditions

offered. They were kept at room temperature in semidarkness during the day and complete darkness at night. Without exception the flies emerged from the pupa cases in due time. Table I. gives the record of this experiment.

TABLE I.

RECORD OF FIRST LOT OF PROGENY FROM *L. sericata* 1912–V.

Series No.	Material Placed in Jar.	No. of Larvæ Placed in Jar Dec. 5, 1912.	No. of Pupæ Formed.				
			Dec. 8.	Dec. 12.	Dec. 16.	Dec. 24.	Jan. 3.
1	Dry sand.	45	22	4	5	12	1 (1 larva dead)
2	Damp sand	45	38 (2 larvæ escaped)	3	1	1	
3	Damp loam	30	13 (loam somewhat dry)	1 (loam dry)	0 (loam dampened)	2	9 (5 larvæ left)
4	Damp sawdust	15	12	(3 larvæ escaped).			
5	Damp cloth	16	4	10 (1 larva dead)	1		

In the first place it is obvious from this table that drying has acted as an inhibitor to pupation while moisture has accelerated it. Thus in Series 1 we notice considerable delay in pupation produced apparently by the dryness of the environment. In Series 2 on the other hand, the damp sand furnished a very favorable condition for pupation. In Series 3 are a few pupations at first, and then with the drying out of the loam, pupation ceases. Moistening the medium again produces pupation. The very favorable conditions in Series 4 and 5 may be due to the fact that the sawdust and cloth were kept very moist. Series 5 shows also that it is not necessary that the larvæ should have a chance to bury themselves in order to pupate, and this I have observed in large numbers of cases in my cultures where the larvæ have readily pupated in wooden boxes as soon as they have left the fish. There is one other thing noticeable here which was not observed in the first experiment and that is that the larvæ in the dry sand although slightly slower in pupating than the others have not entered a condition of indefinite prolongation

of the prepupal period as in the case of 1912-*f*. My only explanation for this is that the temperature being higher in August than in November, drying out had been more rapid at the former time and thus a condition was reached in which the larvæ had not enough moisture to be able to pupate. This was supplied them in the later steps of the experiment.

The following experiment brings out another disturbing factor which acts as an inhibitor to pupation.

One hundred and seventy-six full-grown larvæ were obtained on December 11, 1912, which were also progeny from the later layings of the same *L. sericata* ♀, 1912-*V*. These were put into very damp sand crowded in a glass jar and kept at room temperature. On December 27 it was found that only 36 had pupated. The remaining 140 were distributed in five jars and their subsequent history is recorded in Table II.

TABLE II.

RECORD OF SECOND LOT OF PROGENY FROM *L. sericata*, 1912–*V*.

Series No.	Material Placed in Jar.	No. of Larvæ Placed in Jar Dec. 27, 1912.	No. of Pupæ Formed.				
			Jan. 3.	Jan. 19.	Feb. 5.	Feb. 15.	Mar. 14.
6	Dry sand	30	29	1			
7	Damp sand	30	7	0 (sand dry but dampened again slightly)	6 (sand dry and left so)	2 (19 larvæ including those from series 8 transferred to damp cloth)	19 dead flies found in bottle
8	Very damp sand	30	20	4	2 (sand dry, remaining larvæ put with Series 7)		
9	Dry bottle	30	30				
10	Damp cloth	20	7 (cloth dry and left so).	8	5		

From the delay in pupation from December 11 to December 27 it would appear that the crowding tended to inhibit pupation.

The larvæ were packed in so closely that their movements could be felt by each other and for this reason they were kept active. Upon being separated into smaller lots they soon pupated with the exception of Series 7. For the delay in this case I have no explanation, and it would appear that there are other unknown disturbing factors.

A female specimen of *Calliphora viridescens* (1912-21) was taken in the vicinity of the Bussey Institution, November 20, 1912. Eggs were soon deposited and the larvæ attained full growth on or before December 11. On that date they were placed in dry sand. No pupæ were formed and the larvæ were transferred to damp sand on December 18. Another transfer was made after about one week as pupation had not appeared and the larvæ were placed in dry sand. On January 7 a count was made and there were found to be 3 pupæ, 271 living larvæ, undersized and wrinkled, and 13 dead larvæ, wrinkled and dry. Of the three pupæ, 2 failed to emerge, although flies were formed inside, and the other emerged normally. The larvæ were placed in a jar with very damp cloth. Examination was not again made until January 30 when 2 normal flies were found, 1 ♂ and 1 ♀, 20 dead larvæ, 91 pupæ, and 158 living larvæ. The cloth was still damp. The larvæ were then transferred to a dry glass jar, and by February 15 were all dead and dried up, except that nine misshapen pupæ were formed which did not emerge. Of the other 91 pupæ which were kept on damp cloth only twelve had emerged by February 15, giving six males and six females. The others were dead.

The ill luck in getting these larvæ to pupate may be explained, I think, by the fact that they were at first crowded and thus disturbed each other. This was the condition up to January 7, a period of at least twenty-seven days, when they were observed to be undersized and wrinkled and some of them had died. Apparently in this case we have a condition very different from that of the first experiment recorded in this paper. It is possible that this may be due to the generic difference of the flies as prolongation of the larval stage of *Luciliæ* has often been observed to produce the pinkish coloration of the fat bodies, while in all cases the larger species, *Calliphoræ* and *Cynomyia*, have retained

their white color but have become more contracted. In general the larger species pupate more readily than the *Luciliæ*.

In no case has there been any correlation of abnormalities of chætotaxy either with imperfectly formed flies, or with perfectly formed undersized individuals. My counts include several thousand specimens of various sizes and the lack of any correlation between number of bristles and size has been so obvious that I have made no measurements to establish this principle. I can, however, furnish numerous specimens of full size with greatly reduced chætotaxy and numerous minute specimens having the full number of bristles and even additional ones. Walton,[1] however, from a count of ten specimens of *Belvosia bifasciata* Fabr. concludes that the larger specimens have additional bristles while the smaller individuals are likely to show reduction. As I have not made a study of the parasitic Tachinid flies, with respect to chætotaxy I am unable to pronounce upon the correctness of this conclusion. Number of bristles appears to be an hereditary matter in the blow-flies, Calliphorinæ,[2] and as yet there is no sufficient evidence that environmental factors enter into their determination.

The normal color changes of the fly after eclosion are of interest and may well be described here for comparison with the abnormal conditions. In all the species bred, the fly emerges from the puparium by pushing off the cap by means of the ptilinum. The insect is at first very small and shrunken, but in a few minutes the ptilinum is withdrawn and the tracheæ filled with air. Thus the fly immediately assumes a size much larger than the puparium from which it has just emerged. The color is now white with pinkish and bluish tints, which deepen in a few minutes until at the end of one half hour after eclosion they become deep purple. During the next hour this changes to the normal metallic blue in *Calliphoræ* and *Cynomyia*, the males of the latter genus gradually taking on more or less of a dark greenish color in the course of a few hours. In *Luciliæ* the condition is very different inasmuch

[1] Walton, W. R., '13, "The Variation of Structural Characters Used in the Classification of Some Muscoidean Flies," *Proc. Ent. Soc. Wash.*, XV., 1, Apr., 1913.

[2] Whiting, P. W., '13, "Observations on the Chætotaxy of Calliphorinæ," *Ann. Ent. Soc. of America*, VI., 2.

as the deep purple gives place to bright metallic green which is the color most in evidence at the end of one and one half hours after eclosion. If the fly be anæsthetized with ether the change of pigment is inhibited and the deep purple color may be made to persist throughout life. This does not seem in any way to interfere with the normal activities of the insect.

The assumption of the bronze color in *Lucilia sericata* was made the subject of some investigation and it was found that the factors governing the rapidity of production of this hue were to a great extent of an hereditary nature. Considerable variation occurs among the individuals in regard to the rapidity with which this change from green to bronze occurs and in general it may be said that this takes place in the males more rapidly than in the females. In both sexes, however, the bronze may appear in certain regions of the body before the purple has been replaced by green in other parts and it would appear as if in some cases the bronze followed the purple directly without the intervention of the green. Variation is also considerable as regards the position of the bronzing, in some cases the abdomen becoming bronze while the thorax is yet green, while in other cases the reverse occurs. No evidence is yet found for an environmental cause influencing the rapidity of bronzing, but the process appears to be altogether independent of light and temperature. As regards the latter factor, however, it is desirable that more thorough experiment should be performed under more perfectly controlled conditions. That the chief cause for bronzing, however, is hereditary appears from the following experiment.

A female of *L. sericata*, 1913-*E*, taken near the Bussey Institution, March 19, 1913, produced 39 males and 43 females. Of this lot those that reddened most quickly were selected and a mating was obtained from one pair. This pair produced 70 males and 77 females. A further selection was made from the reddest of these flies, which were examined in less than twenty-four hours after eclosion. One of the pairs selected produced a large family consisting of 366 males and 343 females. At this point the color selection was abandoned, the family being continued as a selection for additional bristles. A rapid effect of selection was noticed through the course of the experiment so that each

succeeding generation averaged much more bronzy in appearance than the preceding. At the close of the experiment in the F_3 generation, properly the second generation of selection, practically all the flies assumed considerable of the bronzy color before they were examined, which was done once every day.

In the selection in the opposite direction the results were not as striking. A female of *L. sericata*, 1913-*F*, was taken near the Bussey Institution, March 19, 1913. She was chosen because she appeared greener than many of the others that were seen about the building. In a few days she deposited eggs from which 24 males and 19 females were reared. Many of these were bronze-colored as soon as they had hardened but a few were green after a period of two or three days with but a slight amount of bronze. A single mating obtained from a pair of these latter gave 93 males and 90 females which averaged much greener than the second generation of the red selection 1912-*E*. At this point the experiment was cut short by the death of the flies selected.

The flies of both the red and the green selections were placed in boxes and allowed to become dry. It was intended to group them into classes according to color and thus to demonstrate more clearly the hereditary nature of the bronzing factors. After the specimens had dried, however, it was observed that the pigment had changed considerably, the reds becoming much greener and the greens often being streaked with blue. It was therefore found necessary to abandon this more accurate proof of the hereditary nature of the bronzing until such time as another selection could be made and the colors of the flies recorded as soon as killed by comparison with standard color charts.

Experiments are now under way which it is hoped will throw more light upon the conditions governing the life histories and habits of these flies.

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